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Evaluating the effects of prolonged peracetic acid dosing on water quality and rainbow trout *Oncorhynchus mykiss* performance in recirculation aquaculture systems

John Davidson[a], Steven Summerfelt[a,1], David L. Straus[b], Kevin K. Schrader[c], Christopher Good[a,*]

[a] The Conservation Fund's Freshwater Institute, 1098 Turner Road, Shepherdstown, WV, 25443, USA
[b] USDA, Agriculture Research Service, Harry K. Dupree - Stuttgart National Aquaculture Research Center, Stuttgart, AR, 72160, USA
[c] USDA, Agricultural Research Service, Natural Products Utilization Research Unit, University, MS, 38677, USA

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ABSTRACT

Peracetic acid (PAA) is an effective disinfectant/sanitizer for certain industrial applications. PAA has been described as a powerful oxidant capable of producing water quality benefits comparable to those expected with ozone application; however, the water oxidizing capacity of PAA in aquaculture systems and its effects on fish production require further investigation, particularly within recirculation aquaculture systems (RAS). To this end, a trial was conducted using six replicated RAS; three operated with semi-continuous PAA dosing and three without PAA addition, while culturing rainbow trout *Oncorhynchus mykiss*. Three target PAA doses (0.05, 0.10, and 0.30 mg/L) were evaluated at approximately monthly intervals. A water recycle rate > 99% was maintained and system hydraulic retention time averaged 2.7 days. Rainbow trout performance metrics including growth, survival, and feed conversion ratio were not affected by PAA dosing. Water quality was unaffected by PAA for most tested parameters. Oxidative reduction potential increased directly with PAA dose and was greater ($P < 0.05$) in RAS where PAA was added, indicating the potential for ORP to monitor PAA residuals. True color was lower ($P < 0.05$) in RAS with target PAA concentrations of 0.10 and 0.30 mg/L. Off-flavor (geosmin and 2-methylisoborneol) levels in culture water, biofilm, and trout fillets were not affected by PAA dosing under the conditions of this study. Overall, semi-continuous PAA dosing from 0.05-0.30 mg/L was compatible with rainbow trout performance and RAS operation, but did not create water quality improvements like those expected when applying low-dose ozone.

1. Introduction

Peracetic acid (PAA) is an antimicrobial agent that is approved for use as a surface disinfectant or sanitizer for various industrial applications including food and beverage operations, organic livestock and crop production, and medical facilities (USEPA, 1993; Warburton, 2014; United States Food and Drug Administration (USFDA, 2015; United States Department of Agriculture (USDA, 2016). In recent years, PAA has also been used to prevent biofilm formation in the paper/pulp industries and as a disinfectant for wastewater treatment (Kitis, 2004). PAA is sold commercially as an equilibrium mixture of acetic acid, hydrogen peroxide, and water, with percent inclusion of ingredients varying among manufacturers. Recently, PAA has emerged as a promising water sanitizer or disinfectant for use in aquaculture, in part, due to its environmentally friendly attributes. When applied at relatively low concentrations, PAA degrades rapidly in aquaculture systems (Pedersen et al., 2009, 2013; Liu et al., 2014) and doesn't form toxic byproducts that could harm fish or create pollution discharge. Only benign compounds are formed during degradation including acetic acid, oxygen, and water (Wagner et al., 2002; Pfuntner, 2011). At present, PAA is approved in Europe for use in veterinary medicine (Lehmann, 1974) and as a water sanitizer for aquaculture systems (Schäperclaus, 1991); therefore, it can be legally used to prevent and control disease outbreaks in fish production systems in the EU. Research carried out over the past decade indicates that PAA can control a variety of fish pathogens including *Icthyophthirius multifilliis* ("Ich") (Meinelt et al., 2007a, b; Meinelt et al., 2009; Straus and Meinelt, 2009; Sudová et al., 2010; Picón-Camacho et al., 2012), *Saprolegni*a spp. (Marchand et al., 2012; Straus et al., 2012; Good et al., 2017a), *Flavobacterium columnare* – causative agent of columnaris (Marchand et al.,

* Corresponding author.
E-mail address: cgood@conservationfund.org (C. Good).
[1] Current work address: Superior Fresh, W15506 Superior Fresh Drive, Hixton, WI54635.

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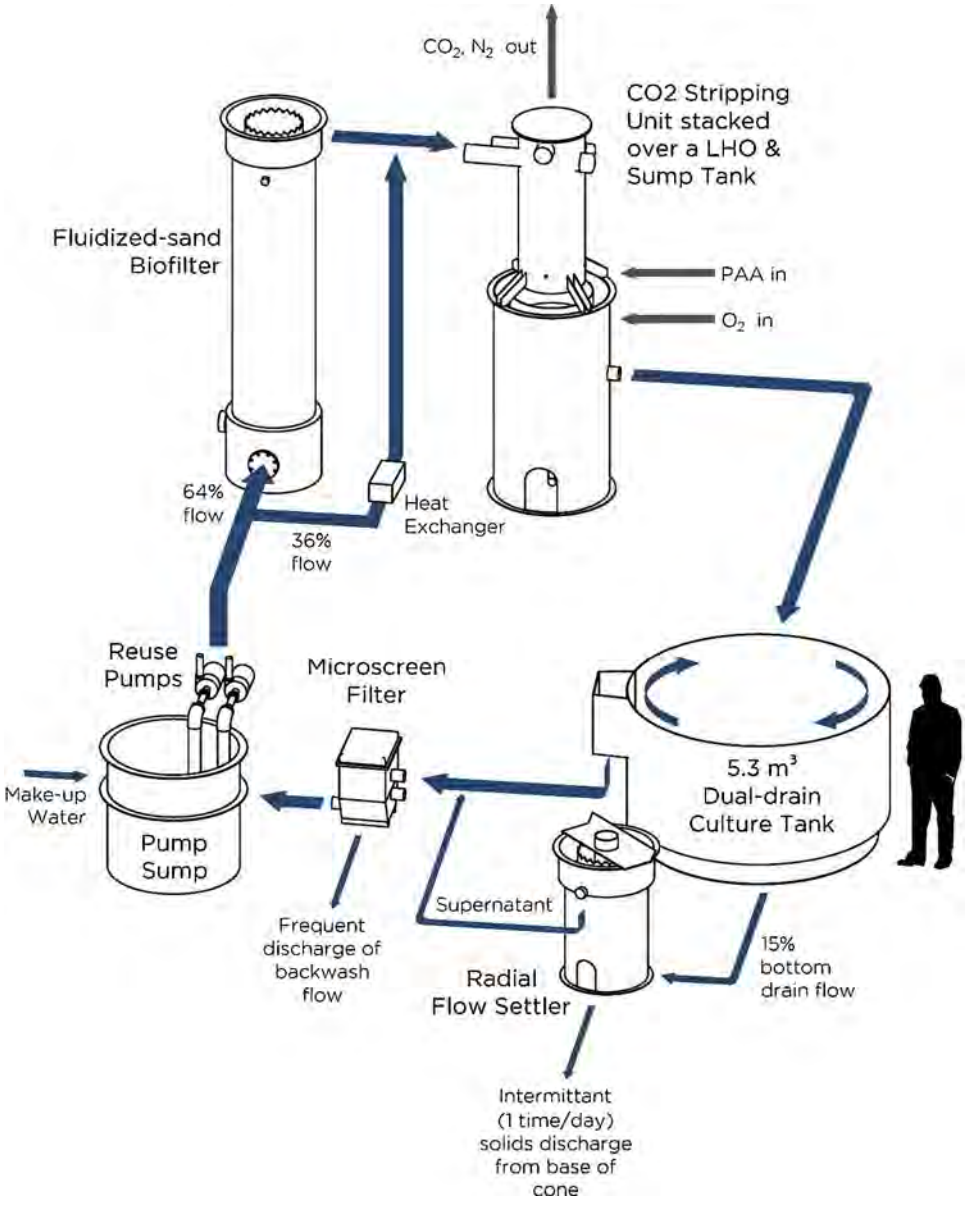

Fig. 1. Water flow, process design, and point of PAA application for an individual experimental reuse system (9.5 m^3) used during the study (courtesy Kata Sharrer, TCFFI Engineering Services).

2012), *Ichthyobodo necatar* (Farmer et al., 2013; Jaafar et al., 2013), *Aeromonas salmonicida* – etiological agent of furunculosis and *Yersinia ruckeri* – causative agent of enteric redmouth disease (Meinelt et al., 2015), and marine microalgae *Tetraselmis chuii* (Liu et al., 2016), among others (Pedersen et al.,2013). However, PAA has not gained approval in the US as a veterinary aid or water sanitizer with fish present. In June 2017, the US Environmental Protection Agency accepted the first registration of a commercial PAA compound (VigorOx SP-15 Antimicrobial Agent, Peroxychem Inc., Philadelphia, PA, USA), which is approved for use in US aquaculture for: (1) sanitizing surfaces of harvesting equipment; and (2) cleaning and disinfecting fish culture tanks when water is drained and fish are absent (Straus et al., 2018).

Most trials demonstrating the effectiveness of PAA for treating or preventing fish disease have been conducted in flow-through systems; however, studies investigating its use in recirculation aquaculture systems (RAS) are limited. Much of the existing research related to the use of PAA in RAS has focused on effects to nitrification and/or the development of standard operating procedures for its application. Pedersen et al. (2009) found that batch addition of PAA to achieve 1 mg/L had minimal effect on nitrification compared to 2 and 3 mg/L PAA, which resulted in significant, prolonged increases of nitrite levels in freshwater RAS culturing rainbow trout *Oncorhynchus mykiss*. A later study determined that increased organic matter content significantly increased PAA decay and noted that biofilm and fish biomass also contributed to its dissipation rate (Pedersen et al., 2013). During another RAS-focused trial, Liu et al. (2017a) evaluated pulse applications of PAA delivered every three to four days over a 5-wk period to achieve a concentration of 2 mg/L PAA in a tank culturing common carp *Cyprinus carpio*. Fish were initially stressed by this procedure as measured by waterborne cortisol but appeared to adapt to subsequent treatments.

There is still much to learn about the use of PAA in RAS and its potential benefits. In particular, a greater understanding of its ability to improve RAS water quality is lacking. The oxidizing capacity of PAA in RAS is of interest because PAA has been described as having properties similar to ozone (Pedersen et al., 2015), a powerful oxidant that is frequently used for RAS water treatment. Ozone improves the culture environment by enhancing water clarity, microflocculating fine solids, adding oxygen, and reducing dissolved metals, nitrite, and organic

concentrations (Summerfelt and Hochheimer, 1997; Davidson et al., 2011; Gonclaves and Gagnon, 2011; Powell and Scolding, 2018). Antimicrobial effects can also be achieved when high ozone doses are applied to RAS water followed by ultraviolet (UV) irradiation (Liltved, 2002; Summerfelt, 2003; Sharrer and Summerfelt, 2007; Summerfelt et al., 2009), and improved growth performance, health, and survival of cultured species have been attributed to the enhanced culture environment created by ozone (Davidson et al., 2011; Good et al., 2011; Powell and Scolding, 2018). However, ozone is relatively expensive and complex to use (Summerfelt and Hochheimer, 1997; Gonclaves and Gagnon, 2011) and can present safety hazards for fish and human health (Gearhart and Summerfelt, 2007); therefore, an alternate water treatment method would be welcomed by the RAS industry.

In addition, research evaluating the effect of PAA on the common off-flavor compounds geosmin and 2-methylisoborneol (MIB) in RAS has not been reported in peer-reviewed literature. The occurrence of off-flavors in RAS-produced finfish products continues to be detrimental to this industry sector (Schrader et al., 2013), as off-flavor can contribute to consumer dissatisfaction, result in a negative perception of aquaculture products, and inhibit future purchasing (Tucker, 2000). Yet, a proven method that immediately mitigates off-flavor in the primary fish production system (grow-out) has not been developed. Most RAS operations employ specific depuration/ "purging" protocols to remove off-flavors from fillets (Davidson et al., 2014a; Lindholm-Lehto and Vielma, 2018); however, this production step results in added capital, operating, and labor costs for the farmer. Given the reported oxidizing capacity and antimicrobial effectiveness of PAA, investigation into its effects on off-flavor-producing bacteria and common off-flavors in RAS is warranted and could provide insight towards a solution to this ongoing problem.

To this end, a study was developed to provide a comprehensive evaluation of the effects of semi-continuously dosed PAA on water quality, rainbow trout performance, and off-flavor compounds in replicate RAS. This work was designed to gain a better understanding of the full benefit of PAA dosing in RAS with intention to add to the body of knowledge on PAA use in aquaculture, inform regulatory decision making related to its use in culture systems with fish present, and to contribute to the development of standard operating procedures for its use in RAS.

2. Methods

2.1. Experimental design

Six replicate RAS, described by Davidson et al. (2009; Fig. 1), were used for the study. Three RAS were semi-continuously dosed with PAA, and the other three RAS did not receive PAA and served as controls. Three target PAA doses (0.05, 0.10, and 0.30 mg/L) were evaluated separately at approximately monthly intervals/dosing periods. The PAA solution used for the trial, VigorOx® SP-15 (PeroxyChem Inc., Philadelphia, PA, USA), consisted of 15–17 % peracetic acid, 9–11 % hydrogen peroxide, 33–38 % acetic acid, and 31–44 % water (PeroxyChem Inc., 2016). Rainbow trout (Troutlodge Inc., Bonney Lake, WA, USA) was used as the test-species.

2.2. Recirculation aquaculture systems

Each 9.5 m^3 RAS recirculated 329 ± 2 L/min (˜87 gpm) of water through a 5.3 m^3 dual drain culture tank, a radial flow settler, a microscreen drum filter with 60 µm screens, a fluidized sand biofilter, a geothermal heat exchanger, a carbon dioxide stripping column, and a low head oxygenator (LHO; Fig. 1). Continuous makeup water flow (2.46 ± 0.04 L/min) originating from a freshwater spring source was added to each pump sump to maintain mean nitrate-nitrogen levels at < 75 mg/L, per recommendation by Davidson et al. (2014b) for onsite rainbow trout production. Makeup water flow rates were calibrated four to five times weekly via bucket testing, and cumulative makeup water addition was measured with digital flow meters installed upstream of float valves which delivered spring water to the systems. The water recycle rate was > 99% on a flow basis, system hydraulic retention time (HRT) averaged 2.7 days (37% of system volume exchanged daily), and mean feed loading rate was 1.38 ± 0.02 kg feed/m^3 of daily makeup water. Tank HRT was approximately 15 min. Sodium bicarbonate (Church & Dwight Co. Inc., Ewing, NJ, USA) was added to each RAS as needed to maintain mean alkalinity levels from 100 to 200 mg/L. Sodium chloride (Diamond Crystal Naturals Solar Salt, Cargill Inc., Minneapolis, MN, USA) was added to each RAS for most of the study to maintain 2–3 ppt salinity as a prophylactic measure against Ich, which was diagnosed, treated, and eliminated via chlorine disinfection at the conclusion of the research trial preceding the present study.

2.3. Peracetic acid dosing

A 208-L (55-gal) drum of VigorOx SP-15 PAA solution was purchased and placed on top of a spill containment pallet. A cooling jacket (Powerblanket, Salt Lake City, UT, USA) receiving a continuous flow of cool (13–14 ° C) spring water was placed around the drum to maintain constant temperature and limit decomposition of the contained solution. A length of stainless-steel conduit was connected and extended through the PAA drum adapter plug to approximately 5 cm from the bottom of the container. Opaque, acid-compatible tubing (Masterflex C-flex L/S #14, Cole Parmer, Vernon Hills, IL, USA) was routed through the conduit to the bottom of the drum and through three pump heads (Item EW-07014-21, Cole Parmer) connected to separate Masterflex L/S peristaltic pumps (Model 07528-10, Cole Parmer) which supplied a semi-continuous dose of PAA to each treatment system. Semi-continuous dosing was achieved by using an on-off pumping cycle (0.5 min on/ 4.5 min off), which was established by integrating a PLC relay (Model SG2-20HR-A, TECO, Taipei, Taiwan) with the peristaltic pump controls. A slow drip of PAA entered the systems at the head space of the LHO distribution plate (Fig. 1).

PAA dosing rate was calculated as follows:

$$\frac{\text{Water Recycle Flow (L/day)} \times \text{Target PAA Concentration (mg/L)}}{10^6 \times 0.15\ (\text{Percent PAA VigorOx SP} - 15)\ \text{day}} = \frac{\text{VigorOx PAA (kg)}}{\text{day}}$$

Daily PAA required (kg/day) was converted to mL/min to establish the dosing rate necessary to achieve the specified target concentration. Dosing rate was validated by collecting drip samples in a graduated cylinder during a stopwatch-timed interval. A room-air monitoring system (Model F12/D Analytical Technology, Inc., Collegeville, PA, USA) was situated nearby the PAA dosing system and wired to trigger an alarm if an unexpected PAA leak caused unsafe off-gas concentrations. This safety measure was adopted due to the American Conference of Governmental and Industrial Hygienists (ACGIH) 2014 establishment of a Short-Term Exposure Limit for airborne PAA of 0.4 ppm (PeroxyChem, Inc., 2016). Three target PAA doses (0.05, 0.10, and 0.30 mg/L) were evaluated separately at approximately monthly intervals/dosing periods. Following the 0.10 mg/L trial, rigorous data collection was temporarily delayed to troubleshoot an unexpected turbidity problem that occurred in RAS from both treatments. During this period, PAA dosing was maintained at a rate that targeted PAA concentrations of 0.10-0.15 mg/L.

2.4. Rainbow trout

Rainbow trout were received as fertilized eyed-eggs, hatched onsite, and cultured in flow-through and partial reuse systems prior to the study. Trout were cultured in the experimental RAS for two and a half

months prior to dosing PAA. To begin the study, each RAS contained approximately 370 rainbow trout (407 ± 6 g), which resulted in an average biomass density of 28.8 ± 0.5 kg/m^3 among replicate RAS. After the 0.10 mg/L PAA dosing period, fish numbers were reduced by approximately 140 fish per RAS to maintain fish densities within acceptable welfare limits defined by the onsite Institutional Animal Care and Use Committee.

2.5. Fish performance sampling

Mortalities were removed and recorded daily to assess cumulative survival. Fish sample size (n) calculated using equations in Bhujel (2008) yielded an impractical number of fish due to the expanded standard deviation expected for trout approaching 2 kg.

n = [(Z * standard deviation)/ accepted error $_g$)]^2; where Z = 1.65 and accepted error = 20 g

Therefore, a correction factor calculation (Martin et al., 1987) was applied to normalize sample size relative to tank population (N).

n* = 1/[(1/n) + (1/N)]

Length and weight measurements of a random sample of 100–110 trout (minimum 27% of the population) from each RAS were collected to begin the study as a baseline, prior to dosing PAA and at the end of each PAA dosing period. Thermal growth coefficient (TGC), condition factor (CF), feed conversion ratio (FCR), and fish survival (%) were calculated using the following formulae:

TGC = ((End Weight ^ (1/3) – Start Weight ^ (1/3))/ ((Days Between * Avg. Temp.) x 1000)

where weight is in grams, length is in mm, and temperature is in º C.

CF = 100,000 * Weight / (Length) ^{3}

FCR = Cumulative Feed Delivered / Fish Biomass Gain

Survival (%) = ((Number Fish to Begin – Cumulative Morts + Culls)/ Number Fish to Begin) *100

2.6. Feeding methods

A constant 24-h photoperiod was provided to facilitate “around-the-clock” feeding and consistent water quality. Rainbow trout in each RAS were fed at the same rate for the first week of the study; thereafter, feeding rates were fine-tuned separately per RAS based on observations of feeding activity and wasted feed. Fish were fed to apparent satiation using a computer operated feeding system (The Conservation Fund Freshwater Institute, Shepherdstown, WV, USA), programmed to deliver short feed bursts once an hour via automated feeders (T-drum 2000CE, Arvotec, Huutokoski, Finland). Feeding rates were reduced accordingly when fish were culled following the 0.10 mg/L PAA dosing trial. A commercially-available 42/16 (protein/fat) trout diet (Zeigler Brothers Inc., Gardners, PA, USA) was fed throughout the study.

2.7. Water quality sampling and analyses

Water samples were collected from the side drain of each RAS, and most parameters were tested onsite according to methods described in APHA (2012) and HACH (2003; 2015) (Table 1). An array of 25 dissolved metals/elements were analyzed by REI Consultants Inc. (Beaver, WV, USA) (Table 1). The effective concentration of PAA stock solution was validated using HACH Company’s application note titled “Determination of Peracetic Acid and Hydrogen Peroxide in Water: Concentration Range of 0.01 to 35% (Titration)”, using a sample volume of 0.2 mL. The PAA concentration of inlet and side drain tank water was determined using HACH Company’s Application Note titled “Determination of Peracetic Acid and Hydrogen Peroxide in Water: Concentration Range of 0.1 to 10 mg/L”, using only the procedure for PAA. Test samples from the ‘Concentration Range of 0.1 to 10 mg/L’ method were analyzed using a DR6000 spectrophotometer (Hach Company, Loveland, CO, USA).

2.8. Off-flavor sampling and analysis

Water, biofilm, and fillet samples were collected at the beginning of the study prior to PAA dosing and near the end of each PAA dosing period for analysis of off-flavor compounds, geosmin and MIB. Glass scintillation vials (20 mL) with foil-lined caps were used for collection of water and biofilm samples. Water samples were collected at the side drain of each RAS by submerging the vials and capping underwater for a complete fill void of air bubbles. Biofilm samples were scraped from the sidewall of culture tanks near the inlet after draining the tank volume by several inches. A small amount of tank water was used to rinse biofilm into each vial. Methods for determination of geosmin and MIB in water and biofilm samples followed Shrader et al. (2013). Specifically, gas chromatograph sample vials were heated at 40° C for 20 min before volatile compounds were absorbed onto a 100-µm polydimethyl siloxane (SPME) fiber (Supelco, Bellfonte, PA, USA). The fiber assembly was shaken for 10 min during the absorption period and desorbed for 2 min at 250° C in the injection port of an Agilent 7890B gas chromatograph-mass spectrometer (GC—MS) (Agilent Technologies, Santa Clara, CA, USA) with a 5977B mass selective detector operated in selected ion monitoring mode. The conditions of the gas chromatograph were as follows: (1) initial oven temperature was 60° C for 0.5 min; (2) then ramp rate of 30° C/min to 100° C; (3) then ramp rate of 20° C/min to 300° C with an isotherm time of 2 min; and (4) the maintenance of flow pressure was at 18 lb/in^2 with helium used as a carrier gas. The molecular ion base peaks were monitored at *m/z* 168, 95, and 135 for MIB and at *m/z* 182, 112, and 126 for geosmin. A DB-5 capillary column (5%-phenyl-methylsiloxane, 30 m, 0.25 mm inside diameter, 0.25-µm film thickness; J&W Scientific, Folsom, CA, USA) was used. The retention time for geosmin was 6.6 min and, for MIB, 5.1 min. Standards for MIB and geosmin were prepared in deionized water at 0.1, 0.5, 1.0, and 2.5 µg/L. The original standards were obtained from Wako Chemicals USA, Inc. (Richmond, VA, USA) and were included at the beginning, middle, and end of each group of samples analyzed using a CombiPal autosampler (LEAP Technologies, Inc., Carrboro, NC, USA).

In addition, three rainbow trout were randomly collected from each RAS near the end of each PAA dosing interval. Trout were humanely euthanized via percussive stunning and filleted. Skinless, right-side fillet portions were packaged in zip-lock freezer bags and frozen prior to shipment for analysis. One 20-g portion from the anterior of each fillet was used to obtain distillate following standard microwave distillation procedures and methods outlined by Lloyd and Grimm (1999). Each distillate sample was analyzed using SPME GC–MS.

2.9. Statistical analysis

Water quality data were analyzed using a mixed models approach that modeled water quality criterion as dependent variables; treatment, time, and treatment x time as independent fixed factors; and RAS/tank as a random effect nested within treatment (Ling and Cotter, 2003; Thorarensen et al., 2015). Analysis of covariance (ANCOVA) with feed loading rate (daily makeup water (m^3)/daily feed (kg)) modeled as a covariate was used to analyze dissolved metals and nutrient concentration data from each PAA dosing trial. Mean off-flavor concentrations and fish performance metrics were analyzed using a Student’s t-test. Each data set was analyzed for normality using a Shapiro-Wilk test. Non-gaussian distributed data sets were analyzed using non-parametric statistics, including the Kruskal Wallis test. A probability level of 0.05 was used to determine significance. Separate analyses were carried out for water quality, performance metrics, and off-flavor for each PAA dosing trial where these data were available. All statistical

Table 1
Water quality parameters evaluated, methodologies, and frequency of testing.

Parameter	Method of Analysis	Frequency of Recording/Testing
Dissolved Oxygen	Hach SC100 Controller & LDO® Probe	Daily
Oxidative Reduction Potential	Hach SC100 Controller & Differential ORP Sensor	Daily
Temperature	Hach SC100 Controller & Differential ORP Sensor	Daily
Salinity	YSI 30 Salinity/Conductivity/Temperature Meter	4-5 times weekly
Specific Conductance	YSI 30 Salinity/Conductivity/Temperature Meter	4-5 times weekly
Alkalinity	Hach Method 8203 - Sulfuric Acid Digital Titration pH endpoint. Accumet #AB150	3 times weekly
pH	Standard Methods 4500-H^+ B – Electrode	3 times weekly
Carbon Dioxide	Hach Method 8223 - Sodium Hydroxide Buret Titration pH endpoint. Accumet #AB150	Once weekly
Biochemical Oxygen Demand	Standard Methods APHA 5210B - 5-day test (No prefiltration) YSI Model 58, YSI BOD probe #5905	Once weekly
Nitrate Nitrogen	Hach Method 8171 - Cadmium Reduction	Once weekly
Nitrite Nitrogen	Hach Method 8507 USEPA Diazotization	Once weekly
Total Ammonia Nitrogen	Hach Method 8038 USEPA Nessler	Once weekly
Total Coliform Bacteria	Hach Method 10,029 - Membrane Filtration, Fisher Isotemp Incubator #516D	Once weekly
Total Heterotrophic Bacteria	Hach Method 8242 - Membrane Filtration, Fischer Isotemp Incubator #516D	Once weekly
Total Phosphorous	Hach Method 8190 – USEPA PhosVer3 with Acid Persulfate Digestion. DRB200 reactor	Once weekly
Total Suspended Solids	Standard Methods APHA 2540D – 1.5 µm filter papers dried at 103-105 ° C. Thelco Oven #6540, Mettler Toledo #AE240 and #PM30K	Once weekly
True Color	Hach Method 8025 - Platinum-Cobalt Standard	Once weekly
Ultraviolet Transmittance	Hach Method 10,054 - Organic UV Absorbing (UV-254)	Once weekly
Dissolved Metals	Inductively Coupled Plasma Atomic Emission Spectrometry	3 events (1 for each PAA dosing rate)

Spectrophotometers DR2700 and DR6000 (Hach Company, Loveland, CO, USA) were used for analysis of nitrate nitrogen, nitrite nitrogen, total ammonia nitrogen, and total phosphorous. Spectrophotometer DR4000 (Hach Company) was used for analysis of true color and UV transmittance.

analyses were carried out using SYSTAT 13 software (2009; San Jose, CA, USA).

3. Results and discussion

3.1. Water quality

3.1.1. Alkalinity

Alkalinity was the only water quality parameter found to be significantly different between treatments during the 0.05 mg/L trial ($P < 0.05$). Mean alkalinity in the PAA-treated and control RAS was 154 ± 1 and 144 ± 2 mg/L, respectively, during this dosing period. Sodium bicarbonate ($NaHCO_3$) was added as needed to maintain alkalinity between 100–200 mg/L; however, $NaHCO_3$ addition was similar between treatments during the 0.05 mg/L trial, i.e., 0.102 ± 0.007 and 0.107 ± 0.003 $NaHCO_3$/ kg feed for the PAA-treated and control RAS, respectively ($P > 0.05$). Although PAA appears to have mildly influenced alkalinity during the 0.05 mg/L trial, a discrepancy of 10 mg/L is not relevant for fish health or biofilter performance (Summerfelt et al., 2015; Boyd et al., 2016). Statistical differences in alkalinity levels were not detected between treatments when evaluating target PAA concentrations of 0.10 and 0.30 mg/L (Table 2).

3.1.2. Oxidative reduction potential

During the 0.05 mg/L trial, mean ORP in the PAA-treated RAS was 248 ± 7 mV compared to 212 ± 13 mV in the control RAS. While a statistical difference in ORP was not identified between treatments during this dosing period, a trend towards significance was evident ($P = 0.072$). When comparing ORP between treatments with time, a highly significant effect was found ($P < 0.001$). There may have been a break-through period of several weeks before PAA residuals resulting from the 0.05 mg/L dose began to fully influence ORP (Fig. 2); thereafter, ORP gradually increased in PAA-treated RAS over the remainder of the dosing period (Fig. 2). The trend for PAA to cause an increase in ORP continued with increasing target concentrations (Table 2; Fig. 2). For example, ORP measured in PAA-treated and control RAS during the 0.10 mg/L trial was 268 ± 12 and 203 ± 8 mV ($P < 0.05$), respectively. Similarly, during the 0.30 mg/L trial, ORP reached 290 ± 2 mV in PAA-treated RAS, while levels in the control RAS were 232 ± 11 mV ($P < 0.05$; Table 2; Fig. 2). This ORP response is like that which is typically observed when applying ozone in RAS (Summerfelt and Hochheimer, 1997; Davidson et al., 2011). During the present study, increasing ORP corresponded with increasing target PAA concentrations, indicating the potential for continuously monitored ORP to track PAA residual concentrations. As such, ORP could be used to monitor and/or control PAA residuals through an integrated on/off control loop with the PAA dosing system, much like the proportional-integral-derivative control strategy used to manage ozone residuals in RAS.

3.1.3. Total suspended solids and bacteria

Peracetic acid did not reduce total suspended solids (TSS) levels in the culture water during any dosing period (Table 2). This result is opposite to the TSS reductions that are expected when applying ozone in fish production systems (Rueter and Johnson, 1995; Summerfelt et al., 1997; Davidson et al., 2011). However, it is important to note that TSS levels measured during the present study were substantially greater and more variable compared to concentrations measured during other onsite trials in the same replicate RAS (Davidson et al., 2011, 2014b). The authors hypothesize that increased TSS levels resulted due to periodic bacterial blooms of an organism identified late in the study as *Flectobacillus roseus* (Larkin et al., 1977; Sheu et al., 2009; Adikesavalu et al., 2015). This bacterium was found to be non-pathogenic to rainbow trout (results presently unpublished) but was seemingly present in large enough numbers to create periodic increases in visual turbidity of the culture water of both treatments. Larkin et al. (1977) reported *Flectobacillus* cell diameters ranging from 0.6 to 1.0 µm and lengths of 1.5–5.0 µm; therefore, at least some of these bacteria would have been captured on the surface of the standard 1.5 µm filter papers used for in-house TSS analysis.

The presence of *F. roseus* created an additional opportunity to evaluate the sanitizing effect of semi-continuous PAA dosing from 0.05-0.30 mg/L. Based on periodic observance of turbid conditions and random spikes in TSS associated with *F. roseus* for both treatments, we conclude that semi-continuous PAA dosing from 0.05-0.30 mg/L did not act as a sanitizer for *F. roseus*. In addition, general heterotrophic bacteria and total coliform counts were not significantly reduced at the tested PAA doses (Table 2). Kitis (2004) reported that a disadvantage of PAA as a disinfectant in the wastewater industry is increased organic content in the effluent caused by the acetic acid component, and the associated potential for microbial regrowth. Semi-continuous dosing was employed during this study as a strategy to limit wide water quality fluctuations in favor of constant conditions; however, this strategy may

Table 2
Water quality concentrations (mean ± standard error) measured in PAA-treated and control RAS (n = 3).

	0.05 mg/L PAA		0.10 mg/L PAA		0.30 mg/L PAA	
	PAA	Control	PAA	Control	PAA	Control
Alkalinity (mg/L as $CaCO_3$)	154 ± 1 *	144 ± 2 *	159 ± 3	154 ± 7	141 ± 5	127 ± 5
Biochemical Oxygen Demand (mg/L)	6.8 ± 2.3	6.8 ± 1.7	10.6 ± 4.0	8.6 ± 3.5	11.7 ± 4.7	11.7 ± 1.7
Carbon Dioxide (mg/L)	9 ± < 1	9 ± < 1	10.7 ± 0.3 *	12.2 ± 0.8 *	14 ± 1	13 ± 1
Dissolved Oxygen (mg/L)	10.2 ± 0.1	9.9 ± 0.1	10.2 ± < 0.1	10.1 ± 0.2	10.2 ± 0.3	10.1 ± < 0.1
pH	7.58 ± 0.02	7.51 ± 0.02	7.54 ± 0.03	7.47 ± 0.06	7.38 ± 0.03	7.39 ± 0.01
Nitrite Nitrogen (mg/L)	0.23 ± 0.04	0.16 ± 0.06	0.19 ± 0.09	0.11 ± 0.03	0.09 ± 0.06	0.05 ± < 0.01
Nitrate Nitrogen (mg/L)	69 ± 3	71 ± 1	64 ± 2	69 ± 3	54 ± 3	64 ± 2
ORP (mV)	248 ± 7	212 ± 13	268 ± 12 *	203 ± 8 *	290 ± 2 *	232 ± 11 *
Salinity (ppt)	2.8 ± 0.1	2.7 ± < 0.1	2.9 ± < 0.1	2.9 ± < 0.1	0.4 ± < 0.1	0.4 ± < 0.1
Specific Conductance (µS)	5.0 x 10^3	5.0 x 10^3	5.2 x 10^3	5.1 x 10^3	3.1 x 10^3	3.1 x 10^3
Total Ammonia Nitrogen (mg/L)	0.58 ± 0.06	0.55 ± 0.02	0.58 ± 0.03	0.64 ± 0.05	0.59 ± 0.03	0.68 ± 0.04
Temperature (° C)	13.7 ± 0.1	13.7 ± 0.1	13.0 ± 0.1	13.0 ± 0.1	13.6 ± 0.1	13.6 ± < 0.1
Total Coliform Bacteria (cfu/100 mL)	2.2 x 10^4	2.9 x 10^4	1.4 x 10^4	7.4 x 10^4	7.4 x 10^3	4.7 x 10^4
Total Heterotrophic Bacteria (cfu/mL)	4.6 x 10^3	3.2 x 10^3	2.5 x 10^3	5.2 x 10^3	4.0 x 10^3	9.6 x 10^2
Total Phosphorous (mg/L)	4.2 ± 0.4	4.1 ± < 0.1	4.0 ± 0.1	4.0 ± < 0.1	3.3 ± 0.3	3.5 ± 0.1
Total Suspended Solids (mg/L)	14.8 ± 9.4	10.9 ± 3.0	16.1 ± 8.0	11.6 ± 4.4	9.3 ± 2.6	7.7 ± 0.7
True Color (Pt Co units)	37 ± 2	40 ± 2	32 ± 2 *	40 ± 2 *	18 ± 1 *	23 ± 2 *
UV Transmittance (%)	69 ± 1	69 ± 1	71 ± 2	69 ± 1	79 ± 1	78 ± 1

* Indicates significant difference between treatments.

have created adaptive conditions for certain microbial populations like that described by Kitis (2004). Likewise, Liu et al. (2017b) reported that continuous application of PAA in flow-through tanks used for rainbow trout culture resulted in excess biofilm formation compared to a pulse application strategy.

3.1.4. True color

Dissolved organic compounds including humic substances originating from soils, sediments, and aquafeeds tend to accumulate in low exchange RAS and likely contribute to the tea-colored water typical of these fish production systems (Christensen et al., 2010; Yamin et al., 2017a). During the 0.10 mg/L PAA trial, true color of the culture water was significantly reduced by PAA dosing ($P = 0.023$). True color in PAA-treated and control RAS was 32 ± 2 and 40 ± 2 Platinum Cobalt (Pt Co) units, respectively, indicating some ability of PAA to oxidize and reduce the dissolved organic compounds responsible for colored water. Water samples analyzed for true color were pre-filtered with 0.45 µm filters to remove solids, which minimized the effect of *F. roseus* on this parameter. True color levels dropped by 50% from approximately 40 to 20 Pt Co units following a reduction of fish numbers and biomass that was carried out after the 0.10 mg/L PAA trial. Due to these changes and the associated reduction in feeding, the concentrations of most water quality constituents, including true color, were reduced.

Overall, these results indicate that PAA, when applied at certain concentrations, has some capacity to oxidize the dissolved organic compounds responsible for tea-colored RAS water. Peracetic acid has been reported to oxidize humic compounds, but at undiluted concentrations used in soil science applications (Schnitzer and Skinner, 1974; Schnitzer and Hindle, 1980). However, the effects of PAA on true color during the present study were not profound, particularly when drawing comparisons to ozone's effect on color. Davidson et al. (2011) demonstrated that application of low-dose ozone (ORP ~ 250 mV) in the same replicate RAS reduced true color by more than 90%, albeit while culturing rainbow trout at greater feed loading rates (3.98 kg feed/m^3 daily makeup water). During another onsite study evaluating the effect of ozone on waterborne hormone levels, ozone reduced true color by 74% from 20 ± 1 to 3.7 ± 0.3 Pt Co units, respectively (Good et al., 2017b), when operating RAS with feed loading rates comparable to the present study. In comparison, true color was reduced by approximately 20 and 22%, respectively, during the 0.10 and 0.30 mg/L PAA trials. Whether or not color reduction and the associated oxidation of dissolved organics and humic substances responsible for colored water is an advantage for Atlantic salmon is unknown. Yamin et al. (2017b) found that common carp exposed to humic substances had lower rates of infection when challenged with *Aeromonas salmonicida*. In addition, several studies have found that humic substances provided a protective

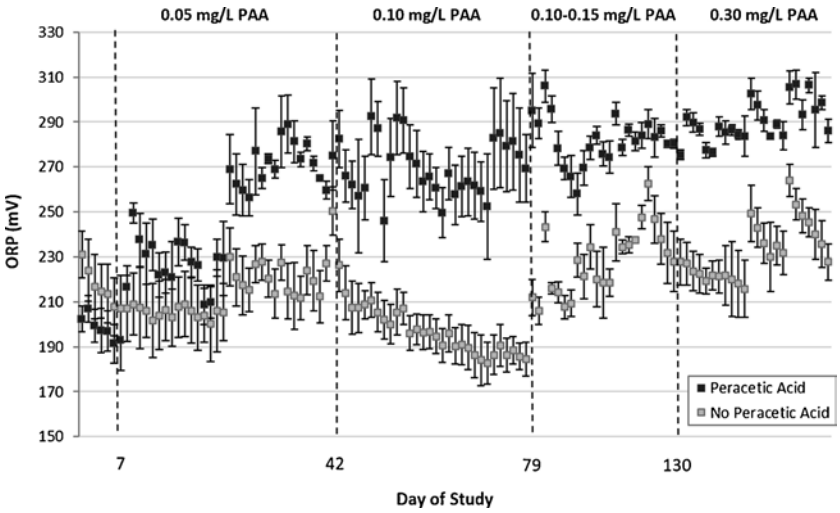

Fig. 2. Oxidative reduction potential (mean ORP (mV) ± standard error) in RAS culture tanks operated with and without peracetic acid dosing over the study duration. Data collection during the 0.10-0.15 mg/L PAA period was limited because of troubleshooting related to culture water turbidity; therefore, time does not reflect exact scale.

effect to the toxicity of dissolved nitrogenous wastes and heavy metals in various fish species (Peuranen et al., 1994; Hammock et al., 2003; Meinelt et al., 2010). On the other hand, clear water void of dissolved organics enhances the ability of fish to see and capture feed, which can result in increased growth (Sigler et al., 1984) and allows the farmer to effectively observe fish (Christensen et al., 2000) health, behavior, and feeding activity. Davidson et al. (2011) reported increased rainbow trout growth in RAS where ozone had significantly reduced color; albeit, other water quality variables were also different between treatments and the growth effect could not be solely attributed to reduced color of the culture water.

3.1.5. Nitrogen

No significant differences in total ammonia nitrogen, nitrite-nitrogen (NO_2-N), or nitrate-nitrogen concentrations were detected during any of the PAA dosing trials (Table 2), indicating that semi-continuous dosing to achieve 0.05-0.30 mg/L PAA did not negatively impact nitrification. A trend for slightly greater mean NO_2-N was evident during all dosing periods, indicating a low-level effect of PAA on nitrification; however, NO_2-N levels remained within safe limits for onsite salmonid production (Davidson et al., 2009). Pedersen et al. (2009) reported that pulse application of 1 mg/L PAA caused minor impacts to nitrification in RAS with submerged biofilters, but PAA levels of 2.0 and 3.0 mg/L resulted in significant and prolonged increases in nitrite. Liu et al. (2017b) also noted that pulse addition of PAA to achieve 1.0 mg/L resulted in partial inhibition of nitrification but found that continuous PAA dosing at 0.2 mg/L did not have a negative impact. The compilation of information regarding the effect of PAA on nitrification suggests that PAA target concentrations from 0 to 1.0 mg/L are compatible with biofilter performance. In considering the effect of PAA dosing on nitrification, the chemical application site is important. During this study, PAA was added at the water distribution chamber of the LHO (Fig. 1) which provided maximum contact time through the water recycle loop for PAA residuals to react and dissipate before reaching the fluidized sand biofilter. A one-time water sampling event during the 0.30 mg/L PAA trial demonstrated that PAA levels dissipated to 0.2 mg/L at the side drain of a fish culture tank. Liu et al. (2017a) used a similar application strategy in RAS by applying PAA at the tank inlet, but with a reduced water flow rate to maximize reaction time, and thereby limit negative impacts on nitrification.

3.1.6. Dissolved metals

Of the 25 dissolved metals/nutrients analyzed in the culture water (Table 3), nine were generally less than the minimum detection limit (MDL) including arsenic, beryllium, cadmium, chromium, cobalt, lead, manganese, molybdenum, nickel, and titanium. During the 0.05 mg/L trial, magnesium, potassium, and sulfur were significantly lower in PAA-dosed RAS. Calcium trended towards significantly lower concentrations in PAA-dosed RAS and iron concentrations were slightly higher ($P = 0.05$) when targeting 0.05 mg/L PAA. No significant differences in dissolved metals concentrations were measured between treatments during the 0.10 mg/L PAA trial; however, dissolved calcium trended towards lower concentrations in RAS where PAA was dosed ($P = 0.05$). Of the dissolved metals evaluated during the 0.30 mg/L PAA trial, boron, iron, and zinc were significantly greater in PAA-dosed RAS ($P < 0.05$).

Overall, the differences in trace metals and nutrients identified between treatments were small in magnitude, i.e., disparities of a fraction of, or only a few mg/L and were not expected to be biologically relevant for fish production. Davidson et al. (2011) found that dissolved copper and zinc were significantly reduced ($P < 0.05$) in the same replicated RAS when applying low-dose ozone (ORP ~ 250 mV). During the same study, dissolved iron was not detected in ozonated RAS compared to the controls when operating with extremely low water exchange rates (HRT > 94 days) and mean feed loading rates of 55.9 kg feed/ m^3 makeup water. Reduction of heavy metals such as copper and zinc due to ozonation was an important finding because these water quality constituents can accumulate in low exchange RAS (Davidson et al., 2009) and are toxic to fish at relatively low concentrations (Spear and Pierce, 1979; United States Environmental Protection Agency (USEPA, 2007; Davidson et al., 2009). During the present study, copper, zinc, and iron were generally unaffected by PAA dosing, except for during the 0.30 mg/L trial where slightly greater concentrations of iron and zinc were measured in the PAA systems. Ultimately, the PAA doses evaluated during this study did not provide an advantage for reduced heavy metals concentrations like that which is expected when applying low-dose ozone.

3.2. Off-flavor

Geosmin and MIB concentrations in water, biofilm, and trout fillets were not affected by PAA during any of the dosing periods ($P > 0.05$). Geosmin concentrations were undetectable (below the instrument detection limit of 1 ng/L) in RAS water and were measured at relatively low concentrations in the biofilm prior to the study, and MIB was not detected (< 1 ng/L) in both the culture water and biofilm (Table 4). Rainbow trout fillets, however, contained substantial concentrations of geosmin and MIB to start, suggesting that trout had bioaccumulated

Table 3
Dissolved metals/trace element concentrations (mean ± standard error) measured in PAA-treated and control RAS (n = 3).

	0.05 mg/L PAA		0.10 mg/L PAA		0.30 mg/L PAA		All Sample Events
Parameters (mg/L)	PAA	Control	PAA	Control	PAA	Control	Makeup Water
Aluminum	0.007 ± 0.001	0.006 ± 0.001	0.009 ± 0.001	0.008 ± < 0.001	< det	< det	0.010 ± 0.003
Barium	0.226 ± 0.025	0.343 ± 0.083	0.261 ± 0.053	0.204 ± 0.007	0.225 ± 0.003	0.213 ± 0.005	0.213 ± 0.004
Boron	0.061 ± 0.007	0.096 ± 0.027	0.075 ± 0.023	0.044 ± 0.002	0.065 ± 0.002 *	0.052 ± 0.002 *	0.040 ± 0.003
Calcium	110 ± 0.3 †	111 ± 0.3 †	121 ± 1 †	125 ± 1 †	117 ± 1	117 ± 1	116 ± 1
Copper	0.031 ± 0.002	0.033 ± 0.001	0.032 ± 0.001	0.031 ± 0.002	0.023 ± 0.002	0.024 ± 0.002	< det
Iron	0.035 ± 0.005 †	0.017 ± 0.002 †	0.053 ± 0.002	0.038 ± 0.012	0.028 ± 0.003 *	< det *	< det
Magnesium	12.6 ± 0.1 *	12.9 ± 0.1 *	13.2 ± 0.1	13.4 ± 0.2	14.3 ± 0.3	14.1 ± 0.2	12.4 ± 0.1
Potassium	19.4 ± 0.2 *	21.2 ± 0.4 *	22.9 ± 0.2	23.6 ± 0.6	11.2 ± 1.0	10.2 ± 0.8	2.2 ± < 0.1
Selenium	0.013 ± 0.002	0.015 ± 0.002	< det	< det	< det	< det	< det
Silicon	5.59 ± 0.02	5.59 ± 0.05	6.05 ± 0.01	6.12 ± 0.02	5.41 ± 0.03	5.34 ± 0.04	5.30 ± 0.19
Sodium	849 ± 7	896 ± 19	832 ± 30	811 ± 9	27 ± 3	26 ± 2	9 ± < 1
Strontium	1.03 ± 0.01	1.04 ± < 0.01	0.99 ± < 0.01	1.01 ± 0.01	1.05 ± 0.01	1.06 ± < 0.00	1.06 ± 0.01
Sulfur	13.1 ± 0.2 *	13.8 ± 0.1 *	17.0 ± 0.1	17.1 ± 0.7	12.6 ± 0.7	12.1 ± 0.4	7.7 ± 0.2
Vanadium	0.011 ± < 0.001	0.011 ± < 0.001	0.003 ± < 0.001	0.003 ± < 0.001	0.003 ± < 0.001	0.003 ± < 0.001	0.006 ± 0.003
Zinc	0.065 ± 0.029	0.143 ± 0.042	0.146 ± 0.042	0.083 ± 0.002	0.119 ± 0.003 *	0.094 ± 0.011 *	0.078 ± 0.005

* Indicates significant difference between treatments ($P < 0.05$).
† Indicates trend towards significance ($P = 0.05$).

Table 4
Geosmin and MIB concentrations (mean ± standard error) in water (ng/L), biofilm (ng/L), and trout fillets (ng/kg) collected at the end of each PAA dosing period from PAA-treated and control RAS (n = 3).

	Baseline		0.05 mg/L PAA		0.10 mg/L PAA		0.30 mg/L PAA	
ng/L; ng/kg	PAA	Control	PAA	Control	PAA	Control	PAA	Control
Geosmin (Water)	< det	< det	21 ± 15	16 ± 3	59 ± 30	54 ± 23	11 ± 5	9 ± 5
Geosmin (Biofilm)	19 ± 4	23 ± 5	2717 ± 2612	3789 ± 2916	394 ± 311	3895 ± 3836	185 ± 133	236 ± 157
Geosmin (Fillets)	1831 ± 604	544 ± 35	3096 ± 1824	4951 ± 1569	8449 ± 4038	2757 ± 775	3546 ± 2057	2431 ± 1182
MIB (Water)	< det	< det	17 ± 10	10 ± 1	8 ± 4	27 ± 23	3 ± 1	2 ± 0
MIB (Biofilm)	< det	< det	51 ± 35	209 ± 137	43 ± 19	186 ± 12	14 ± 7	23 ± 7
MIB (Fillets)	101 ± 11	87 ± 32	343 ± 222	607 ± 79	400 ± 323	1303 ± 1281	55 ± 10	27 ± 5

these off-flavors in a separate production system. During the study, concentrations of geosmin and MIB generally increased in the culture water and biofilm, and tended to persist or, in some cases, increase in fish flesh. The substantial drop in geosmin and MIB in RAS water and biofilm during the 0.30 mg/L trial is interesting but occurred in PAA-treated RAS as well as control systems, indicating that other factors influenced concentrations of these off-flavor compounds (e.g., reduction of daily feed amounts). Ultimately, the present study indicated that semi-continuous dosing of PAA in RAS to achieve target doses of 0.05-0.30 mg/L does not reduce geosmin and MIB concentrations in water, biofilm, or fish flesh, and therefore does not mitigate these types of off-flavor problems.

A challenge with applying oxidants in RAS with the intention to mitigate off-flavor problems is that these compounds must be dosed to produce low residual concentrations that are compatible with fish health and nitrification. As such, oxidant residuals are only present in the water from the point of application (during this study at the LHO) through the culture tank, with nearly full dissipation taking place before the recycle flow reaches the biofilter. The lack of impact of oxidants such as low-dose ozone (Davidson et al., 2011) and PAA on off-flavors in RAS could, in part, be related to their effectiveness being limited to a section of the water recycle loop. Biofilms inside of pipes and unit processes such as the drum filter, heat exchangers, and biofilter are sources of geosmin and MIB-producing bacteria (Schrader and Summerfelt, 2010) that likely remain untreated. In addition, the dosing approach used during the present study may have promoted biofilm growth, which is contrary to conditions that are consistent with reduced concentrations of geosmin and MIB in fish fillets (Davidson et al., 2014b). Research by Liu et al. (2017b) possibly corroborates this theory, as this manuscript reported that continuous, low dose application of PAA enhanced biofilm formation in flow-through tanks stocked with rainbow trout. Conversely, Lindholm-Lehto et al. (2018) recently noted that batch addition of PAA to achieve 2.2 mg/L PAA in RAS raising rainbow trout resulted in a significant reduction of geosmin and MIB, particularly with increased frequency of batch addition. Although PAA does not appear to be a viable solution for eliminating common off-flavor problems in RAS under the conditions of the present study, future work investigating its effect when applied using once daily or periodic batch addition and/or semi-continuously at greater concentrations may be necessary to fully understand its potential for managing common off-flavors.

3.3. Trout performance

Rainbow trout growth curves established for fish from PAA-treated and control RAS overlapped almost identically throughout the study (Fig. 3); therefore, rainbow trout growth was not affected by semi-continuous PAA dosing at the tested target concentrations. During the final dosing period (0.30 mg/L PAA), a small, but insignificant separation in mean fish weights occurred (Fig. 3). Mean rainbow trout weights at study's end for PAA-treated and control RAS were 1911 ± 30 and 1954 ± 11 g. Mean thermal growth coefficient

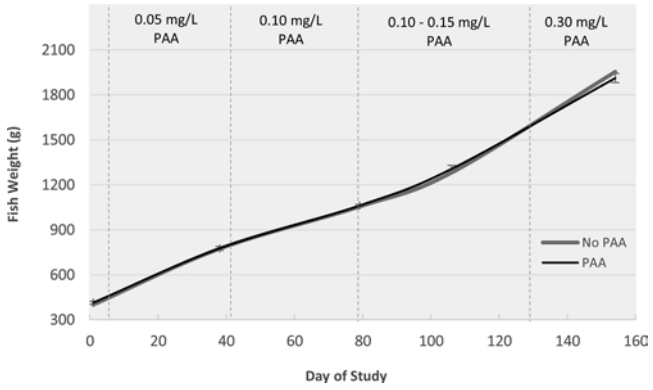

Fig. 3. Rainbow trout growth (mean weight ± standard error) in RAS operated with and without peracetic acid dosing over the study duration.

assessed over the duration of the trial for treatments with and without PAA was 2.41 ± 0.01 and 2.45 ± 0.02, respectively ($P > 0.05$). No significant differences were detected for a variety of other performance responses including feed conversion ratio, condition factor, and fish survival during each PAA dosing period and over the duration of the study (Table 5). Therefore, semi-continuous PAA dosing from 0.05-0.30 mg/L did not negatively affect trout performance and appeared to be compatible with rainbow trout production in RAS under the conditions of this study.

3.4. Costs

Improvements to RAS economics are necessary to enhance the commercial viability of this growing aquaculture sector, including optimization of capital and operating cost efficiencies, fish production capacity, economies of scale, and marketing (Losordo and Westerman, 1994; De Ionno et al., 2006; Liu et al., 2016). However, cost efficiencies specific to individual water treatment technologies have not been extensively studied. The relative costs of PAA application in RAS have not been assessed; however, economic estimates and discussion have been provided for PAA use in different fish production systems and for the wastewater treatment industry. Pedersen and Henriksen (2016) estimated that it would cost a flow-through Danish trout farm (130 L/sec with low organic matter) $20 USD per day for semi-continuous dosing of PAA to achieve prophylactic water treatment, plus the upfront costs for pumps and dosing equipment estimated at $400-500 USD. Kitis (2004) noted the minimal capital investment associated with use of PAA for wastewater treatment, an advantage that likely extends to aquaculture applications. During the present study, the energy and labor costs for effective operation were minimal and the upfront capital costs to setup the PAA dosing system totaled approximately $6000 USD. Capital costs were related to the purchase of peristaltic pumps and associated parts, pump tubing, a spill containment pallet, a room air monitoring system, one 208-L PAA drum, and a drum cooling jacket. These upfront costs may vary depending on water flow to treat and

Table 5
Fish performance metrics (mean ± standard error) to begin the study and at the end of each PAA dosing period for PAA-treated and control RAS (n = 3).

	Baseline		0.05 mg/L PAA		0.10 mg/L PAA		0.30 mg/L PAA	
Fish Performance Metrics	PAA	Control	PAA	Control	PAA	Control	PAA	Control
Fork Length (mm)	289 ± 1	290 ± 1	328 ± 2	330 ± 2	359 ± 1	360 ± 1	425 ± 2	427 ± 5
Weight (g)	414 ± 10	401 ± 6	776 ± 13	773 ± 12	1054 ± 9	1061 ± 8	1911 ± 30	1954 ± 11
Condition Factor	1.9 ± 0.04	1.8 ± 0.02	2.1 ± 0.04	2.1 ± 0.01	2.3 ± 0.01	2.3 ± 0.01	2.4 ± 0.01	2.5 ± 0.02
Feed Conversion Ratio	–	–	1.3 ± 0.02	1.4 ± 0.09	2.2 ± 0.16	1.9 ± 0.08	1.4 ± 0.08	1.5 ± 0.16
% Body Weight Feed	–	–	2.6 ± 0.06	2.5 ± 0.06	2.0 ± 0.07	2.1 ± 0.01	1.5 ± 0.03	1.6 ± 0.02
Biomass Density (kg/m^3)	28.5 ± 0.7	29.1 ± 0.7	52.1 ± 1.5	53.3 ± 0.8	66.3 ± 1.8	62.0 ± 4.8	80.4 ± 2.0	80.6 ± 1.8
Survival (%)	–	–	99.4 ± 0.3	99.1 ± 0.2	96.9 ± 0.5	93.9 ± 3.8	97.8 ± 0.9	98.5 ± 0.4

decisions related to equipment purchasing for worker safety. The 2018 cost for one 208-L drum of VigorOx SP-15 PAA is $950 plus shipping or a 1249-L tote can be purchased for $6150 plus shipping. Consistent with Kitis (2004), the relative cost of the PAA chemical itself is relatively high; however, the potency of PAA has shown to be 100 times that of hydrogen peroxide (Straus et al., 2012). Based on the dosing regimen used during the present study, semi-continuous peracetic acid treatment of a water recycle flow of 329 L/min in one 9.5 m^3 RAS would require the purchase of one 208-L drum every 7–8 months; however, treatment of the water recycle flow of an onsite semi-commercial scale RAS (~4000 L/min recycle flow; 270 m^3 total volume) would require a new PAA drum every 18–19 days or a new 1249-L tote every 3–4 months. During the 0.30 mg/L PAA trial, for which these cost estimates are based, only marginal water quality benefits were observed. As such, fish farmers using RAS most likely would not adopt semi-continuous PAA dosing as a feasible strategy for broad-ranging water quality improvement. However, these cost estimates may be relevant for other PAA application strategies in RAS such as once-daily or periodic batch addition, which has shown promise related to antimicrobial treatment effects (Good et al., 2017a; Liu et al., 2017a, 2017b), or semi-continuous dosing in flow-through systems where prophylactic effects have been reported (Pedersen and Henriksen, 2016). To the authors' knowledge, a detailed cost assessment for ozonation in RAS has not been carried out; therefore, accurate cost comparisons cannot be drawn. Several publications have reported that the equipment and operating costs associated with ozonation are not trivial, particularly when ozone is applied in combination with UV (Summerfelt and Hochheimer, 1997; Gonclaves and Gagnon, 2011), and the use of ozone comes with its own set of safety considerations for fish and human health (Gearhart and Summerfelt, 2007). Nevertheless, the extensive water quality benefits created by ozone (Davidson et al., 2011; Gonclaves and Gagnon, 2011; Powell and Scolding, 2018) likely justify the capital and operating expenses of this technology.

3.5. Conclusions

The findings from this study indicate that semi-continuous PAA dosing at target concentrations of 0.05-0.30 mg/L in research-scale RAS provided marginal water quality improvements, unlike the profound and wide-ranging enhancements to the fish culture environment expected when applying low-dose ozone. Nevertheless, these findings do not negate the potential for PAA to be applied differently in RAS, such as with once-daily or periodic batch addition, an approach which has shown promise for antimicrobial and prophylactic control. A comprehensive assessment of water quality, salmonid performance, and off-flavor compounds may be informative when applying PAA in RAS using batch addition up to 1.0 mg/L or with semi-continuous doses to achieve concentrations > 0.30 mg/L.

This research demonstrated a safe and effective protocol for dosing PAA in RAS of this design that could be replicated elsewhere. The injection point for PAA dosing to each RAS was strategically identified. By adding the PAA drip just above the LHO distribution plate, a mixing effect was provided by water cascading through the carbon dioxide stripping column. This location was also ideal because it followed the fluidized sand biofilter, thereby allowing ample reaction time to limit the effect of PAA residuals on nitrification. The authors recommend this point of PAA application for future studies evaluating PAA use in RAS. In addition, PAA residuals appear to impact oxidative reduction potential readouts like ozone, indicating that ORP could be used as an on/off control when applying PAA semi-continuously or otherwise as an indirect measure for PAA residual concentrations when using alternate dosing methods.

Although semi-continuous dosing of PAA from 0.05-0.30 mg/L did not result in profound water quality improvements, this dose was compatible with fish health and performance, as well as biofilter operation. These results are important when considering PAA's capacity as a water sanitizer or disinfectant and its possible use to improve fish health by controlling pathogens in the water column. Although state of the-art RAS inherently provide robust biosecurity against the introduction of obligate fish pathogens, opportunistic pathogens will occasionally cause disease in RAS under conditions that favor the infectious agent (Wedemeyer, 1996). Given the innocuous nature of low concentration PAA demonstrated during this study, PAA should not be ruled out as a viable chemical to control pathogenic bacteria with fish present in RAS. Few chemicals are compatible as water sanitizers in RAS; therefore, more research is required to understand PAA's antimicrobial effects in RAS at low concentrations and when applied using methods other than those used during the present study.

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